Naval Surface Warfare Center
Carderock Division
West Bethesda, MD 20817-5700

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Survivability, Structures, and Materials Department

Technical Report

Disinfection of Water by Ultrasound: Application to Ballast Water Treatment

by

Robert A. Brizzolara, Eric R. Holm, and David M. Stamper

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Ultrasound has potential application in disinfecting a variety of water streams, including shipboard ballast water to avoid transfer of non-indigenous species between geographic locations. Two approaches for improving the performance of ultrasound in disinfecting bacteria were examined: 1) optimizing the ultrasonic intensity by varying the treatment cell diameter, and 2) using ultrasound in conjunction with a second treatment. A contact time for one log kill of an E. coli pure culture of 0.6 minutes was measured when using higher average intensities resulting from reduced treatment cell diameters, a substantial improvement over previous work. Combined treatment consisting of ultrasonic and thermal treatment resulted in a reduction of about 40% in contact time for one log kill of E. coli. Since a contact time of 0.6 minutes per log kill is still likely to be too long for a flow-through treatment system for ballast water, the applicability of ultrasound to ballast water treatment is expected to focus on zooplankton, for which ultrasound is very effective. A second treatment that targets the bacteria could also be employed. Additionally, ultrasound is effective in disinfecting both bacteria and zooplankton in lower flow rates and may have application to other water treatment applications. Additional experimentation is recommended using ultrasound to disinfect natural seawater.

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ADMINISTRATIVE INFORMATION

The work described in this report was performed at Naval Surface Warfare Center, Carderock Division (NSWCCD), West Bethesda, MD in the Survivability, Structures and Materials Department (Code 60) by personnel in the Materials (Code 61) and Environmental Quality Divisions (Code 63). The work was funded by the Office of Naval Research (ONR), Arlington VA (Code 332) as part of the "Advances to Ship-borne Waste Treatment Processes by Application of High-Power Ultrasound" task of Program Element 0602747N.

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EXECUTIVE SUMMARY

Ultrasound has potential application in disinfecting a variety of water streams, including shipboard ballast water to avoid transfer of nonindigenous species between geographic locations. Two approaches for improving the performance of ultrasound in disinfecting bacteria were examined: 1) optimizing the ultrasonic intensity by varying the treatment cell diameter, and 2) using ultrasound in conjunction with a second treatment. A contact time for one log kill of an E. coli pure culture of 0.6 minutes was measured when using higher average intensities resulting from reduced treatment cell diameters, a substantial improvement over previous work. Combined treatment consisting of ultrasonic and thermal treatment resulted in a reduction of about 40% in contact time for one log kill of E. coli. Since a contact time of 0.6 minutes per log kill is still likely to be too long for a flow-through treatment system for ballast water, the applicability of ultrasound to ballast water treatment is expected to focus on zooplankton, for which ultrasound is very effective. A second treatment that targets the bacteria could also be employed. Additionally, ultrasound is effective in disinfecting both bacteria and zooplankton in lower flow rates and may have application to other water treatment applications. Additional experimentation is recommended using ultrasound to disinfect natural seawater.

INTRODUCTION

Ultrasound has long been known to be capable of disinfecting water. Ultrasound causes cavitation bubbles to form and then violently collapse, killing single-celled organisms by cellular disruption and disintegration. Lower frequencies (20-50 kHz) are generally more effective than higher frequencies (>100 kHz), although higher frequencies more efficiently produce reactive free radicals (Mason, 2000). Sonolytic inactivation of *Escherichia coli* was demonstrated and the effects of frequency and power characterized (Hua and Thompson, 2000). These experiments were performed at power intensities ranging from 4.6 to 74 W/cm^2, total power ranging from 80 to 140 W, power per volume of 0.27-0.46 W/ml, and frequencies of 20, 205, 358, 618 and 1071 kHz. The most effective frequency for inactivation was 205 kHz. The inactivation rate increased with intensity. 26 kHz ultrasonic energy had a germicidal effect against *E. coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*, fungus and viruses (Scherba et al., 1991). Mortality increased with both exposure time (in minutes) and power. 20 kHz ultrasound applied using a 60 W transducer decreased viability of *E. coli* exponentially with time (Allison et al., 1996). Higher intensity also increased the rate of cell mortality (Furuta et al., 2004).

Ultrasound has potential applicability in disinfecting a variety of water streams, including potable water and wastewater. Of particular interest in this report is the application of ultrasound to the treatment of shipboard ballast water to avoid the transfer of nonindigenous species from one geographic location to another. Ballast water exchange, the replacement of water in a ship's ballast tanks, is currently the most widely used method of treating ballast water. During ballast water exchange, ballast water taken aboard in coastal areas is replaced with ocean water while the vessel is in transit between ports. The purpose of the exchange is to remove from the ballast

tank coastal organisms originating in the departure port, and replace them with oceanic organisms, which may not survive when released in the coastal or fresh waters of the destination port (National Research Council, 1996). Thus, the process of exchange is not necessarily intended to alter the concentration of organisms in a tank, but instead to affect the species structure of the tank community. Results to-date suggest that exchange has highly variable effects on the abundance (as opposed to the community structure) of zooplankton, phytoplankton and bacteria found in ballast water (for example, Locke et al., 1991; Smith et al., 1996; Dickman and Zhang, 1999; Zhang and Dickman, 1999; Wonham et al., 2001; Drake et al., 2002).

Given that exchange may cause no predictable reduction in the concentration of organisms in ballast water, it may be an unacceptable treatment option in view of recently proposed regulations for ballast discharge. The International Maritime Organization's (IMO) new International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO, 2004) sets discharge standards on ballast water based on the abundance of organisms, and not whether they are coastal or oceanic in origin. In the United States, Senate bill S. 363 (the Ballast Water Management Act of 2005) takes a similar approach, but sets discharge concentrations that are as much as two orders of magnitude lower than the IMO standards. There are other difficulties with the exchange approach: 1) some ships remain close to shore, so they cannot exchange ballast in the open ocean, 2) the design of some ballast tanks makes efficient exchange impossible, and 3) mid-ocean exchange cannot be performed in higher sea states without endangering the ship. Given the issues with exchange, if the proposed regulations or others like them are adopted, the shipping industry will require treatment systems that efficiently remove or inactivate all or nearly all organisms resident in ballast water.

A number of approaches or technologies for treatment of ballast water have been considered or evaluated, including thermal techniques (for example, Rigby et al., 1999; 2004), deoxygenation (Mountfort et al., 1999; Tamburri et al., 2002), ultraviolet irradiation and filtration/separation (Sutherland et al., 2001; Waite et al., 2003), advanced oxidation techniques (Cooper et al., 2002), and ultrasonic systems (Mesbahi, 2004). None of these potential solutions are in wide use; treatment systems combining ultraviolet irradiation with filtration have been installed on a small number of ships. It is not known whether any system now available will consistently and efficiently meet the discharge requirements of developing regulations.

We previously evaluated the applicability of high-power ultrasound to the treatment of ballast water in beaker-scale and pilot-scale experiments (Brizzolara et al., 2006, Stamper et al., 2006). Contact times and energy densities were feasible for treatment of zooplankton such as *Artemia*, in the context of flow rates for the ballast water application; however, contact times and energy densities were orders of magnitude higher for bacteria such as *E. coli*. The contact time necessary to reduce the concentration of *Artemia* by one order of magnitude (1 log kill) was found to be 0.5 seconds and the energy density was 0.4 J/ml. For *E. coli*, these values were 1.4 minutes and 180 J/ml. In addition, in the beaker-scale experiments, it was found that the measured contact time for *E. coli* depended linearly on the treated liquid volume. This indicates that the treatment container size in these experiments was larger than the disinfection zone. Put another way, this result indicates that the ultrasonic intensity in at least a portion of the beaker was insufficient to kill *E. coli.*

The effectiveness of ultrasound against bacteria might be improved by the use of combined treatment systems in which ultrasound is used in conjunction with a second treatment. Although these treatment approaches may not be feasible for disinfection of bacteria in ballast water treatment when used singly, the combination of treatment methods can result in a synergy between the two treatments that results in a much greater disinfection performance. Burleson et al. (1975) examined the effects of sonication alone, ozonation alone and sonication combined with ozonation, on various organisms with public health significance including *Staphylococcus aureus, Pseudomonas fluorescens, Salmonella typhimurium,* enteropathogenic *E. coli*, *Vibrio cholerae* and *Shigella flexneri*. Treatment by sonication alone did not inactivate the microorganisms. While ozonation inactivated organisms in secondary effluent from a wastewater treatment plant, simultaneous ozonation and sonication reduced the contact time required. Dahi (1976) found that application of ultrasound enhanced the disinfection effect of ozone on *E. coli*. Phull et al. (1997) found that the use of ultrasound in the 20-800 kHz frequency range reduced the amount of chlorine necessary to disinfect *E. coli*. For chlorination combined with sonication, the order in which the two treatments were applied was important. Sonication prior to chlorination produced a greater kill, because chlorine added before sonication was removed by solution degassing. In addition, sonication of the solution prior to chlorination significantly increased the effectiveness of chlorination, relative to chlorination without sonication. Similarly, ultrasonic treatment at 20 kHz, 150 W reduced the heat resistance of *Bacillus subtilis* spores (Garcia et al., 1989). While sonication followed by thermal treatment reduced the spores' heat resistance somewhat, simultaneous sonication and thermal treatment reduced the temperature required to kill the spores by 5 – 10 °C. Other combined treatment approaches include ultraviolet (Blume & Neis, 2004), propylene oxide and ethylene oxide (Boucher et al., 1967), and glutaraldehyde (Sierra and Boucher, 1971). Thus, for bacteria at least, the application of ultrasonic energy simultaneously with a second treatment might result in benefits such as reduced energy requirements or the ability to meet environmental regulations on water discharges compared to those obtained with the existing treatment technology used alone.

Given the long exposure times required for disinfecting bacteria relative to zooplankton, we examined two approaches for improving the performance of ultrasound in disinfecting bacteria: 1) optimizing the intensity of the ultrasound by optimizing the diameter of the treatment cell, and 2) combining treatment approaches using ultrasound in conjunction with a second treatment. It was hypothesized that reducing the diameter of the treatment cell would improve the exposure times for bacteria by exposing them to a higher average intensity within the treatment cell. For bacteria, combined treatment methods employing ultrasound and a second method may offer a route to disinfection of large water volumes and flow rates. Thermal disinfection damages bacteria in part by softening the cell wall. Therefore, we investigated whether combining ultrasound and thermal treatment would synergistically enhance this effect due to the shear forces the cavitation generates on the bacterium. Combined ultrasound and thermal treatment was also investigated for zooplankton. Similarly, we investigated whether application of ultrasound prior to treatment of bacteria with chlorine would provide damage to the bacterial cell wall via cavitation-induced shear forces, increasing the effectiveness of chlorine. Finally, we investigated the effect of pressure on the effectiveness of ultrasound in killing bacteria.

The objective of this work is to determine the contact time and energy density requirements for ultrasound treatment, alone and in combination with either heat, pressure or chlorination, of

certain ballast water-relevant organisms. These results are intended to guide development of possible ballast water treatment technologies. In addition, the results of this work can be applied to the use of ultrasound for other water treatment applications. A unique aspect of this work is that by measuring the contact time and energy density required to kill a particular organism at a particular ultrasonic intensity, estimates can be made of the size and energy requirements of a full-scale treatment cell. Two different transducer materials were used in this work. Two of the devices in this investigation utilized the magnetostrictive material, TERFENOL-D, whereas another device utilized the piezoceramic material PZT. For a comparison of the two materials, see Moffet et al. (1991) or Bright (2000).

MATERIAL AND METHODS

ULTRASONIC TREATMENT SYSTEMS

Beaker-Scale, Simple Horn Apparatus

The beaker-scale system has been described previously (Brizzolara et al., 2006). The ultrasonic transducer (Etrema Products, Inc., Ames, IA), based on TERFENOL-D magnetostrictive material, drove a 13.3 cm long titanium horn with a circular terminal face having an area 1.26 cm^2. Coolant was supplied to the transducer as low-pressure airflow (house air) during operation. The ultrasonic intensity at the face of the horn is properly determined from the ultrasonic power that the transducer delivers to the water, rather than the electrical power provided to the transducer (as is occasionally done in the literature). Calorimetry is a common method for measuring ultrasonic power (Mason, 1991; Mason, 2000). Energy was measured continuously during experiments, rather than estimation based on average power and exposure time. Ultrasonic power, intensity, and energy in this text are presented as calorimetric values, *i.e.* as energy imparted to the medium. Ultrasonic intensity in the beaker-scale system ranged from 10-30 $W \cdot cm^{-2}$.

Bench-Scale, Cascade Horn Apparatus

The bench-scale, cascade horn system was capable of flow-through or loop-recirculation ultrasonic treatment. The system was fabricated by Etrema Products, Inc. and is shown schematically in Figure 1 and in a photograph in Figure 2. The Branson 900 BCA ultrasonic transducer (based on PZT) and controller (Branson Ultrasonics Corp., Danbury, CT) were mounted to a custom titanium cascade horn (Etrema Products, Inc.) with 3 antinodes with a total surface area of 25 cm^2. Transducer output was logged by a universal serial bus (USB) device and the data downloaded onto a personal computer after the experimental runs. The relative power could be controlled in real time using the digital readout on the controller. The horn was inserted into a custom stainless steel, water-jacketed flow cell (Etrema Products, Inc.). The inside diameter of the flow cell could be modified by insertion of stainless steel sleeves to obtain horn-to-sidewall clearance in this flow cell of 0.4, 0.2, or 0.1", corresponding to treatment volumes of 318, 178 and 136 ml. The 0.4" clearance is equivalent to that on the pilot-scale ultrasonic treatment system described previously (Brizzolara et al., 2006). Pumping through the system was accomplished with a Nemo Model NM015/12 progressive cavity pump (Netzsch Inc., Exton, PA), with ¾" plumbing, valves, pressurization fittings, and sample ports. Temperature control was achieved by circulating chilled (5-10°C) water through the jacketed flow cell and an in-line heat exchanger, and a thermostat-controlled inline heater allowed for testing at elevated temperatures. Due to heating from the ultrasound, temperatures could be controlled from 30-50°C. The system could be sealed and pressurized up to 50 psi with bottled gas (N_2). Sampling required a minimum of 5 psi to recharge the accumulator. Total system volume, with no sleeves in the flow cell, was 2.03 liters; an "accumulator" allowed removal of 200 ml of samples without affecting the plug flow of the medium. The flow rate for the majority of bench-scale cascade horn system experiments was 3.8 $liter \cdot min^{-1}$.

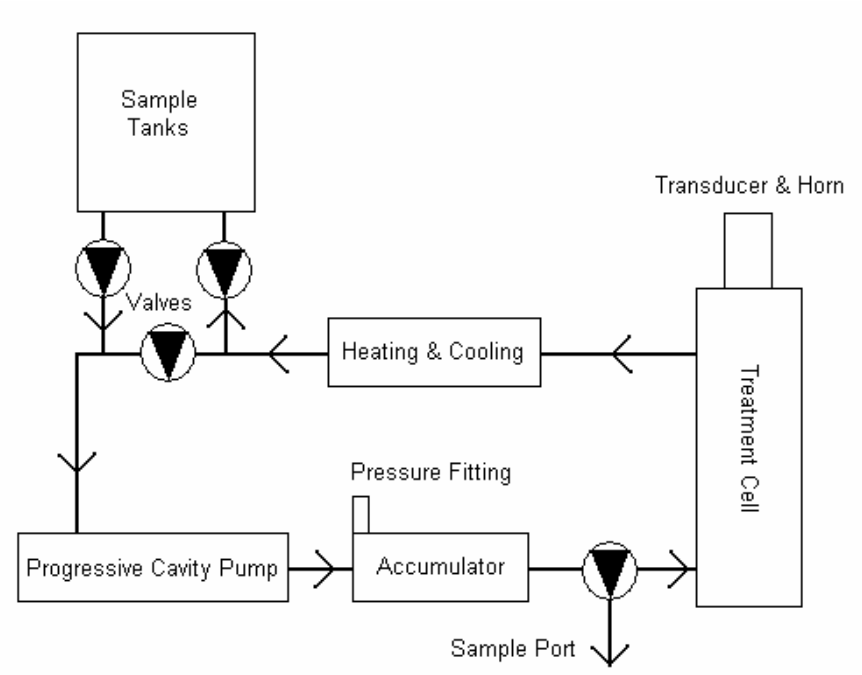

Figure 1. Schematic of bench-scale cascade horn system showing the important components. The device could be set for loop-recirculate or single-pass treatment.

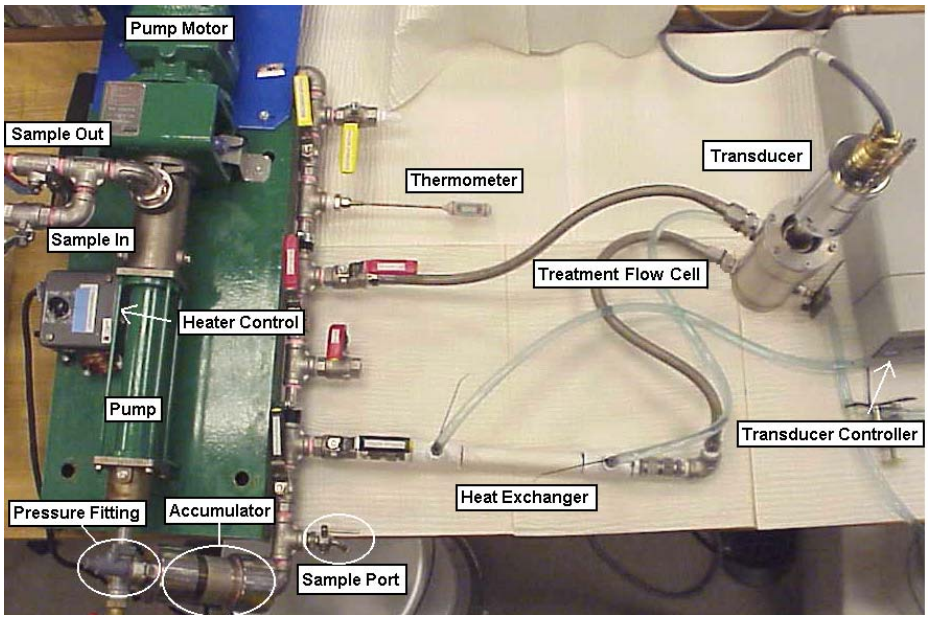

Figure 2. Photograph of bench-scale cascade system.

BACTERIAL CULTURE CONDITIONS

Escherichia coli (ATCC 11775) and *Vibrio cholerae* (ATCC 15748) were grown to mid-log phase (A_{600} = 0.5-0.8) at room temperature (20-25°C), with shaking or magnetic stirring, in trypticase soy broth (Sigma Aldrich, St. Louis, MO). Cells were harvested by centrifugation, washed twice in phosphate buffered saline (pH 7.3, Sigma Aldrich, St. Louis, MO), resuspended in the sonication medium (described below), and allowed to rest for two hours before exposure to high power ultrasound. The cells were treated in this way to approximate stationary phase.

Cultures were allowed to recover from test conditions for 2-3 hours prior to enumeration by dilution plate counting. Control experiments were treated comparably. Serial 10-fold dilutions of samples were made in buffer, spread in duplicate onto trypticase soy agar (Sigma Aldrich) plates, and incubated overnight at 35°C. Duplicate plates with 30-500 colonies were deemed suitable for counting.

ANIMAL CULTURE CONDITIONS

Brine shrimp (*Artemia* sp., www.brineshrimpdirect.com) were raised from eggs in the laboratory, following standard procedures. Artificial seawater (25 ‰) for rearing was prepared from deionized water and sea salts (Instant Ocean; Aquarium Systems, Inc., Mentor, OH). Tests employed nauplii that were less than 24 hours old.

EFFECT OF TREATMENT CELL DIAMETER ON ULTRASONIC DISINFECTION OF BACTERIA

The effect of treatment cell diameter on the efficacy of bacterial disinfection was investigated by inserting sleeves into the treatment cell to reduce its diameter. Treatment cell diameters producing horn-to-sidewall spacings of 0.4, 0.2, and 0.1" were tested. Power and pressure for these experiments were either 275 W (10 psi) or 385 W (10 and 20 psi).

COMBINED TREATMENT APPROACHES FOR DISINFECTION OF BACTERIA

Beaker-Scale, Simple Horn System: Ultrasound and Heat Treatment of Bacteria

For the beaker-scale bacterial disinfection experiments, ultrasound was typically applied to 100 ml of stationary phase *V. cholerae* in a #9850 jacketed sonochemical reaction vessel (ACE Glass, Vineland, NJ). The titanium horn, with a 1.26 cm^2 circular face, was inserted sufficiently (0.5-1.0 cm) to avoid entraining air into the liquid during operation. *V. cholerae* was inoculated to 2-5·10^6 colony forming units per ml (CFU·ml^{-1}) in filter-sterilized synthetic seawater (35 g·l^{-1}, Sigma Aldrich) as the sonication medium. The salinity of this solution was determined independently to be 29‰ (parts per thousand) salinity, as measured with a salinity meter (Model YSI 30, YSI Environmental Co., Yellow Springs, OH). The pH was 8.1-8.2. This sonication medium was aerated by overnight shaking in a sealed bottle with 25% headspace since dissolved gases are known to be important for ultrasonic cavitation (Kondo & Kano 1988; Hua & Thompson 2000).

Temperatures from 25-60 °C were tested by recycling water from a temperature-controlled water bath through the jacket of the reaction vessel. The temperature was monitored using a thermocouple probe. With higher ultrasonic intensity, the temperature was not completely controlled, but the temperature range was recorded. Heat-only control experiments were performed similarly, but without power to the ultrasound device.

Bench-Scale Cascade Horn System: Ultrasound/Heat and Ultrasound/Pressure

The configuration of this system made experimentation with the pathogenic *V. cholerae* impractical, so only *E. coli* was tested. *E. coli* required multiple passes to achieve an energy density of 150 J/ml. Except where noted, experiments were performed at 380 - 390 W (calorimetric), measured as described above. For experiments that combined ultrasound with thermal treatment, 2.5litersof synthetic seawater was heated to 5°C above the target temperature and circulated for a minimum of 10 minutes to allow for aeration of the medium. The treatment cell was used with no sleeves inserted, resulting in a 318 ml volume. Once the temperature reached the desired value, *E. coli* was added to $2 \cdot 10^6$ CFU·ml^{-1} and allowed to mix for another two minutes. After this mixing, the ultrasound unit was turned on, and the system was closed and pressurized to 10 psi. The disinfection of *E. coli* was tested by combining ultrasound with temperatures of 30, 35, 40, and 45 °C at the 318 ml treatment cell volume. The effect of pressure from 5-30 psi was independently tested at 45°C at the 318 ml treatment cell volume.

Chlorination and Ultrasound Treatment of Bacteria

Chlorine was added as NaOCl (bleach) and measured using a modified DPD-FAS (N, N-diethyl-p-phenylene-diamine – ferrous ammonium sulfate) method (Standard Methods, 1989) with a digital titrator and total chlorine pillows (Hach Company). This methodology measures "total chlorine", including chloramines. Since synthetic seawater contains both bromide and iodide that are oxidized by Cl_2 and react on an equimolar basis with Cl_2, what is reported as total chlorine may more appropriately called total residual oxidants.

The possible synergistic effects between ultrasound treatment and chlorination disinfection were studied using *V. cholerae* grown, harvested, and prepared in synthetic seawater as described above. *V. cholerae* was enumerated by dilution plate counting as described above. The $Ct_{99.9}$ (concentration and time to kill 99.9%) for *V. cholerae* was determined at 0.23 and 0.4 mg total $Cl_2 \cdot l^{-1}$. In order to look for synergy, *V. cholerae* exposed to either high energy density or low energy density ultrasound was subsequently exposed to low (less than 0.5 mg·l^{-1}) concentrations of Cl_2. All experiments employed ultrasonic treatment prior to Cl_2 treatment, given that ultrasound can remove Cl_2 from solution by degassing (Phull et al., 1997).

For the low energy density experiment, 550 ml of *V. cholerae* in synthetic seawater was exposed to 12 W·cm^{-2} ultrasonic intensity for 10 minutes, for an energy density of 17 J·ml^{-1}. Ultrasound treated and untreated samples were immediately exposed to 0-0.25 mg·l^{-1} total chlorine in synthetic seawater. The samples were quenched after 1 minute by 1/10 dilution in 0.2% sodium thiosulfate in buffer and the *V. cholerae* enumerated.

A higher intensity and energy density experiment was performed to complement the lower energy density experiment. For this experiment, 100 ml of *V. cholerae* was exposed to

30 $W \cdot cm^{-2}$ ultrasound in the beaker-scale system for 3 minutes, totaling 68 $J \cdot ml^{-1}$. Aliquots were drawn at 0, 1, 2, and 3 minutes of ultrasound exposure for enumeration and for subsequent exposure to chlorine. Bleach was added to 0.23 mg total $Cl_2 \cdot l^{-1}$ in the several samples. Samples were withdrawn at 15 and 30 seconds and then immediately quenched by 1/10 dilution in 0.2% sodium thiosulfate in buffer.

ULTRASOUND AND THERMAL TREATMENT OF ZOOPLANKTON

Initial trials with *Artemia* suggested that testing under static conditions in small (less than 100 ml) volumes would be inappropriate due to the very short contact times required to generate significant levels of mortality. Testing sequential application of ultrasound and heat, using a flow-through reactor, proved unworkable. We therefore examined the effects of ultrasound and heat applied simultaneously, in large volumes. Ultrasound was applied to a 916 ml suspension of *Artemia* in artificial seawater (25 - 28 ‰), in a jacketed beaker (1000 ml, #5340, ACE Glass). The jacketed beaker was filled with 900 ml of aerated (for 24 hours) artificial seawater heated to the appropriate temperature, with 16 ml of a dense solution of *Artemia* added. Temperatures from 30 – 43°C were maintained by recycling water from a temperature-controlled water bath through the jacket of the reaction vessel. Temperature was monitored using a thermocouple probe.

Immediately after addition to the jacketed beaker, *Artemia* were exposed to ultrasound at two intensities – 10 and 20 W/cm^2. As in previous experiments the titanium horn was inserted to a depth sufficient to minimize entrainment of air during operation. Control experiments subjected the *Artemia* to the appropriate thermal conditions, but without power to the ultrasound device. All experiments except for the controls were conducted in duplicate or triplicate.

SAMPLING AND ANALYSIS

Bacteria

Subsamples were typically drawn over the course of a given experiment at five time points. The bacteria were allowed to recover for two hours prior to serial dilution, duplicate plating on trypticase soy sugar (TSA), and overnight incubation. Counts of $CFU \cdot ml^{-1}$ were plotted against time on a semi-log plot and a regression line was applied to produce a kill curve.

Artemia

Subsamples (2 ml) of the 916 ml test volume were drawn at 6 time points: 0.5, 1, 1.5, 2, 3, and 4 minutes after commencement of exposure to ultrasound. Subsamples were dispensed to test tubes and evaluated either immediately after termination of all replicates of the experiment, or 24 hours later in cases were heat treatment caused visible, nonfatal stress (e.g. heat coma). Quantification of live *Artemia* was carried out using a dissecting microscope, with individuals scored as ‘live,’ ‘moribund,’ or ‘dead’ following criteria described in Cooper et al. (2002). The logarithm of the number of live animals per ml was plotted against exposure time and a regression line applied to produce a kill curve.

Determination of Decimal Reduction Time and Decimal Reduction Energy Density

The decimal reduction time (DRT) is defined as the time of exposure of an organism to ultrasound necessary to achieve one log kill. The DRT was calculated from the slope of the kill curve (m), as DRT = $-m^{-1}$. Regression analyses were carried out using the SAS software package (SAS Institute, Cary, NC). In the case of single-pass treatment, the exposure time of organisms to ultrasound was determined from the volume of the treatment cell divided by the flow rate. In the case of recirculation experiments, the exposure time of organisms to ultrasound was determined from the product of the run time and volume of the treatment cell, divided by the volume of the system. The decimal reduction energy density (DRED - $J \cdot ml^{-1}$ per log kill) is defined as the ultrasonic energy density in the treatment cell necessary to achieve one log kill of an organism and is equal to the product of the ultrasonic power and DRT, divided by the liquid volume in the treatment cell. The DRED was calculated similarly by plotting $CFU \cdot ml^{-1}$ against energy density ($J \cdot ml^{-1}$), and statistical analyses were performed using SAS (SAS Institute).

RESULTS AND DISCUSSION

ULTRASONIC DISINFECTION OF BACTERIA: EFFECT OF TREATMENT CELL DIAMETER

Previous experimental work showed that DRTs for ultrasonic disinfection of bacteria are very long, on the order of minutes (Brizzolara et al., 2006). Hence, we investigated whether a smaller treatment cell diameter that produces a higher average ultrasonic intensity within the treatment cell would improve (decrease) the DRT. If the average intensity in the treatment cell is too low, the measured DRT contains two contributions: (1) the time it actually takes to kill an organism at a given intensity, and (2) additional time due to the fact that all bacteria are not treated simultaneously because there is insufficient intensity at all locations in the treatment cell to produce cell mortality. Sleeves were inserted into the flow cell that restricted the horn-to-sidewall clearance from 0.4 to 0.2 and 0.1". The resulting treatment cell volumes were 0.318, 0.178 and 0.136 liters. The flow rate was maintained at 3.8 liters·min^{-1}.

Figure 3 shows the DRT results from several experiments at different power outputs and pressures. The results show that the smallest diameter treatment cell has the shortest decimal reduction time. The fact that the decimal reduction times do not show any sign of leveling off as the treatment cell volume is decreased indicates the possibility that even smaller diameter treatment cells would result in yet shorter DRTs. In other words, the threshold average intensity has not been reached, even at the smallest treatment cell diameter tested. The minimum DRT measured in these experiments was 0.6 minutes – at 385 W and 10 psi. The negative control, which consisted of operating the flow system with the ultrasound turned off, yielded a DRT of 97 minutes. Thus the flow system and pump had little, if any, effect on *E. coli* mortality. The decimal reduction energy densities are shown in Figure 4 and ranged between 100 and 140 J/ml. Given the size of the 95% confidence intervals in this plot, it is difficult to discern whether there is a trend in DRED as a function of treatment cell diameter.

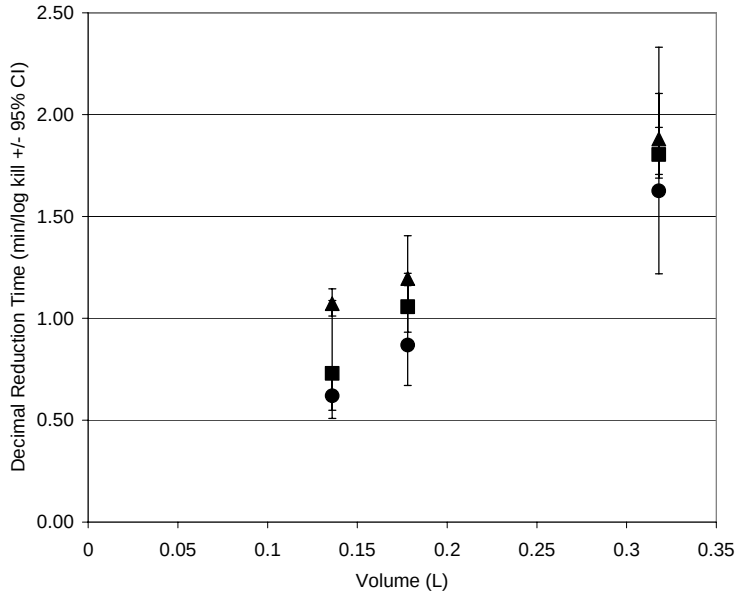

Figure 3. The effect of treatment cell volume (and horn-to-sidewall clearance) on the decimal reduction time of *E. coli* in the bench scale cascade horn system. Treatments were: 380-290 W and 10 psi (●), 380 – 390 W and 20 psi (■) and 270-285 W and 10 psi (▲). DRT was measured to be 97 minutes with ultrasound turned off (negative control).

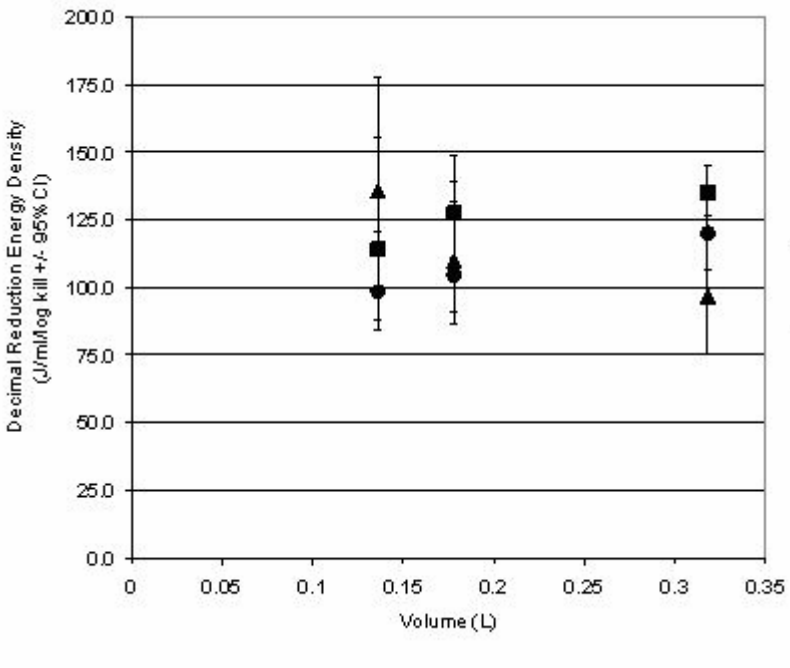

Figure 4. The effect of treatment cell volume (horn-to-sidewall clearance) on the decimal reduction energy density of *E. coli* in the bench-scale cascade horn system. Treatments were: 380-390 W and 10 psi (●), 380-390 W and 20 psi (■), 270-285 W and 10 psi (▲).

DISINFECTION OF BACTERIA WITH ULTRASOUND AND HEAT

The beaker-scale experiments with *V. cholerae* show a clear relationship between the decimal reduction time, energy density, and temperature (Table 1). For intensities of 23-24 W/cm^2, at higher temperatures, *V. cholerae* requires less exposure time and energy density to achieve a 90% reduction in viability. The minimum intensity in the beaker-scale system was 10 $W \cdot cm^{-2}$, below which cavitation could not be sustained. Whether any disinfection could occur without cavitation was not tested.

The 50°C heat only control experiment was similar to the 50°C with ultrasound exposure (Table 1), with no improvement in kill afforded by the ultrasound. Although the control exposure to 50°C was quickly lethal to *V. cholerae*, exposure to 45°C for 10 minutes found no measurable kill. These results show a synergy between heat and ultrasound between 40 and 45°C, given no kill of *V. cholerae* from exposure to 45°C and the ability of *V. cholerae* to grow at 40°C. Warm, sub-lethal temperatures clearly enhance the disinfection capability of ultrasound at the beaker scale.

The effect of heat and ultrasound was also tested using the bench-scale cascade system with *E. coli*. As with *V. cholerae*, a modest, sub-lethal increase in temperature enhanced the disinfection provided by ultrasonic treatment. The decimal reduction times (Figure 5) and energy densities (Figure 6) are significantly lower at higher temperatures with ultrasound at 45°C killing *E. coli* 40% faster and with lower energy density than at 30°C. Regression analyses were carried out using SAS (SAS Institute). Exposure of *E. coli* to 45°C in the test system, as a negative control resulted in a decimal reduction time of 97 min (range 73-143 min).

Taken together, the results from the *V. cholerae* and *E. coli* experimentation show a clear synergy between ultrasound and thermal treatment. It is important to note that these calculations necessarily ignore the energy involved in heating the water ($4.8\ J \cdot ml^{-1} \cdot °C^{-1}$). If the energy used to heat the sonication medium was taken into consideration, the synergy would be eliminated. However, in practical application, the use of heat and ultrasound could involve recycling of thermal energy that would otherwise be wasted (for example, from the ship's propulsion system) into the sonication medium, making this more energy efficient than ultrasound alone.

Table 1. Beaker-scale ultrasound and heat treatment of *V. cholerae*

Temp (°C)	Intensity ($W \cdot cm^{-2}$)	Decimal Reduction Time, mean and range ($min \cdot 100\ ml^{-1}$)	Decimal Reduction Energy Density, mean and range ($J \cdot ml^{-1}$)[a]
32-39 (35[b])	24	2.3 (2.0-2.7)	40 (35-46)
39-44 (40[b])	24	1.9 (1.6-2.3)	33 (28-41)
43-46 (45[b])	23	1.6 (1.0-3.9)	28 (18-73)
45	15	4.0 (3.5-4.5)	40 (33-50)
50	14	0.6 (0.4-1.1)	6.3 (4.5-10.5)
45 control	0	15.3-16.6	N.A.[c]
50 control	0	0.5	N.A.

[a] Energy from ultrasound only, not for heating

[b] Nominal (or target) temperature

[c] Not applicable

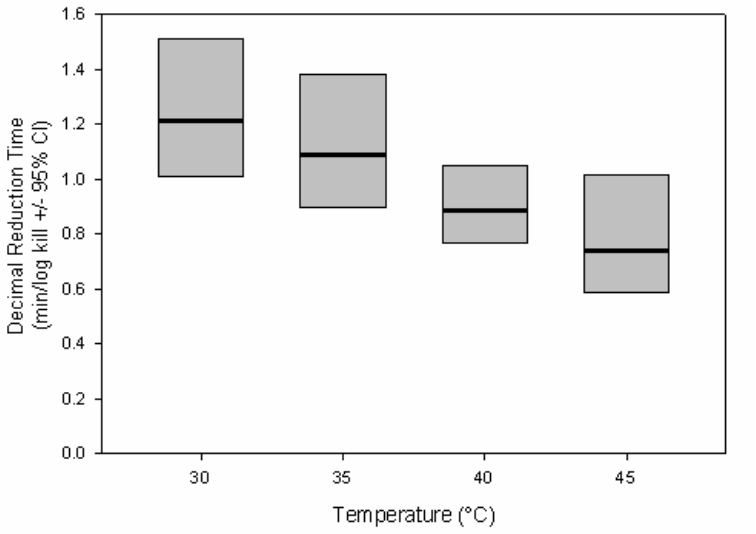

Figure 5. Effect of combined heat and ultrasound on the decimal reduction time of *E. coli* in the bench-scale cascade horn system. The experiments were performed using the largest volume treatment cell (318 ml), a pressure of 10 psi and an ultrasonic power of 380-390 W. These data combine the results from two different runs, showing the mean and 95% confidence interval range for those results.

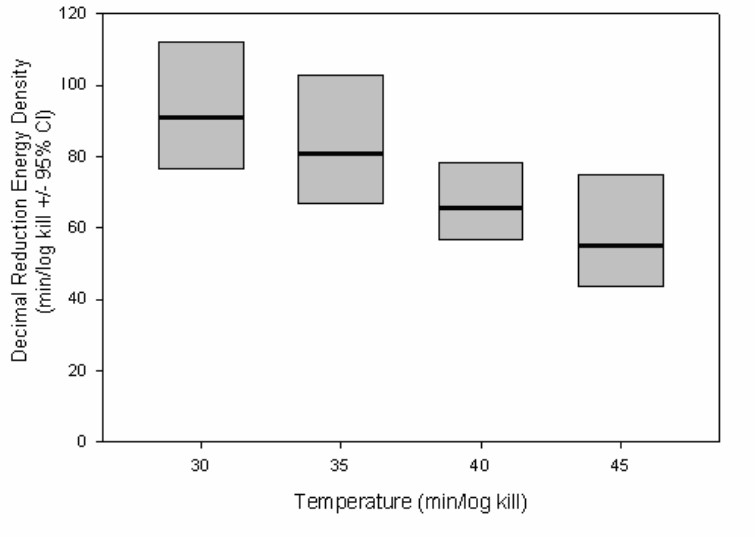

Figure 6. Effect of combined heat and ultrasound on the decimal reduction energy density of *E. coli* in the bench-scale cascade horn system. The experiments were performed using the largest volume treatment cell (318 ml), a pressure of 10 psi and an ultrasonic power of 380-390 W. These data combine the results from two different runs, showing the mean and 95% confidence interval range for those results.

DISINFECTION OF BACTERIA WITH ULTRASOUND AND PRESSURE

Given that ballast water pumping involves an increase in pressure, the effect of pressure along with ultrasound and heat was investigated. Pressure, by itself, was not seen to enhance the kill rate of *E. coli* on the bench-scale cascade system (Figures 7 and 8). Pressure can affect the function of the transducer and horn, however. Higher pressure increases the load on the horn and, depending on the design of the control software and hardware, can demand increased power output for the same horn displacement. For our experiments, the power output was kept constant. This resulted in smaller horn displacement at higher pressures. This differs from the experiments by Pagán et al. (1999) where the horn displacement increased with pressure. They claim a synergy between ultrasound and pressure, but the power increase that necessarily accompanied the increase in displacement with pressure was not noted. In another study, the group did find a synergy between ultrasound and pressure (from 0-300 kPa) at constant horn amplitude (Raso et al., 1998). Although it is difficult to compare various results in the literature due to different equipment and parameters, the evidence in the literature for synergy between ultrasound and pressure is very limited.

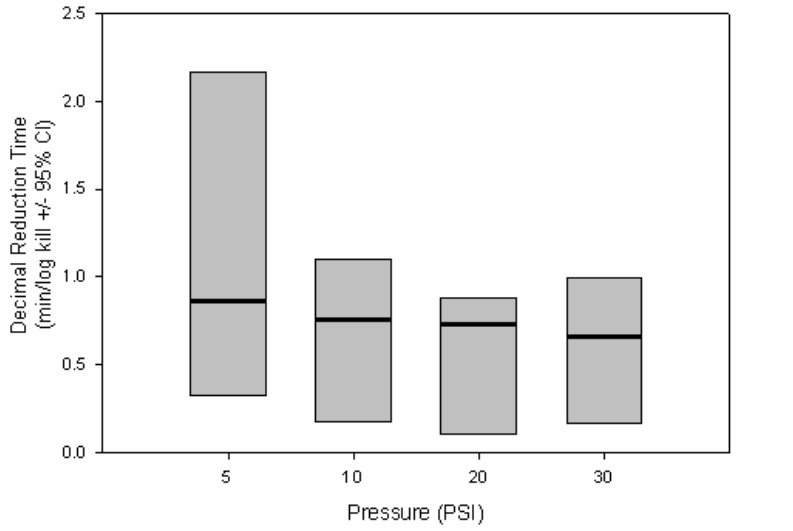

Figure 7. Effect of pressure on the decimal reduction time of *E. coli* in the bench-scale cascade horn system. The experiments were performed using the largest volume treatment cell (318 ml), a temperature of 45°C and an ultrasonic power of 380-390 W. These data combine the results from two different runs, showing the mean and 95% confidence interval range for those results.

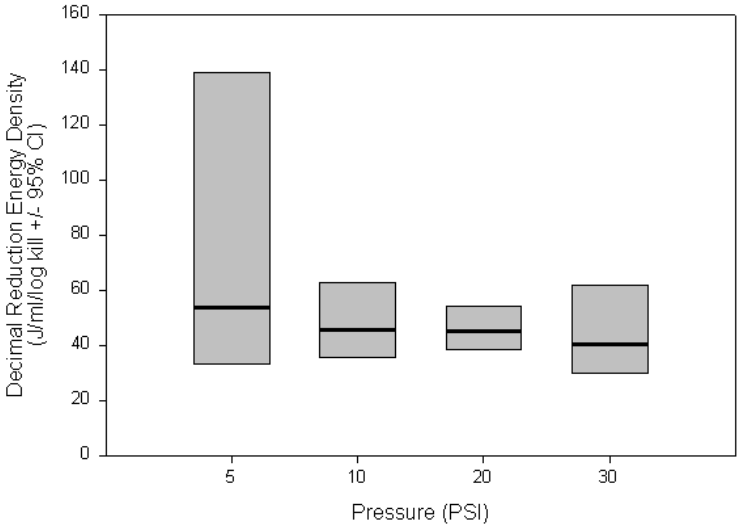

Figure 8. Effect of pressure on the decimal reduction energy density of *E. coli* in the bench-scale cascade horn system. The experiments were performed using the largest volume treatment cell (318 ml), a temperature of 45°C and an ultrasonic power of 380-390 W. These data combine the results from two different runs, showing the mean and 95% confidence interval range for those results.

DISINFECTION OF BACTERIA WITH ULTRASOUND AND CL_2

Given the analytical difficulties of working with sub-ppm concentrations of Cl_2 and the fact that ultrasound can drive Cl_2 from solution (Phull et al., 1997), simultaneous treatment of *V. cholerae* with ultrasound and Cl_2 was judged to be impractical. It was verified that quenching with sodium thiosulfate made total Cl_2 undetectable, with no measurable toxicity from sodium thiosulfate (data not shown).

Figure 9 shows the survival of *V. cholerae* exposed to a low energy density of ultrasound (17 $J \cdot ml^{-1}$). These lightly treated cells were subsequently exposed (within 15 minutes) to increasing concentrations of Cl_2 for 1 minute, along with cells that had not been exposed to ultrasound, as shown in Figure 10. The kill from Cl_2 was similar in these two groups. Chlorine, by itself, was rapidly lethal to *V. cholerae*. As a matter of practical application, if chlorination were applied to disinfect ballast water, very low concentrations should be very effective against planktonic *V. cholerae*, an organism of concern. This absence of synergy suggests that ultrasound treatment does not damage cells such that Cl_2 is able to be a more effective disinfectant. Other researchers, however, have demonstrated that ultrasonic treatment is able to modify suspended (planktonic) cell permeability to hydrophobic compounds (Rapoport et al., 1997) and antibiotics (Pitt et al., 1994; Fitzgerald et al., 1998). Ultrasound has also been demonstrated to induce a (genetic) global stress response in cells that remain alive (Vollmer et al., 1998). In a similar application, ultrasonic treatment was able to enhance antibiotic effectiveness against an *E. coli* biofilm (Rediske et al., 2000), presumably by disrupting the biofilm much as ultrasound is able to disrupt chains, clumps, and flocs of bacteria (Huhtanen, 1968; Phull et al., 1997; Neis & Blume, 2003; Stamper et al., 2006). These previously published results are all consistent with ultrasound being able to damage cells without killing them. Our data, however, do not show any sub-lethal damage from ultrasound contributing to disinfection from Cl_2.

A high energy density ultrasound exposure experiment complemented the low energy density experiment. Samples of *V. cholerae* were exposed to 30 $W \cdot cm^{-2}$ ultrasound for 0, 1, 2 or 3 minutes (0, 23, 46, and 68 $J \cdot ml^{-1}$, Figure 11) and subsequently exposed to Cl_2 (Figure 12). *V. cholerae* that experienced higher exposures to ultrasound with subsequent treatment with Cl_2 experienced less mortality than samples exposed to the Cl_2 treatment alone (Figure 12). Since ultrasound kills bacteria by lysing them, the cell contents liberated by ultrasound treatment may have reacted with Cl_2 and protected the surviving cells from Cl_2 exposure. Thus, combining ultrasound and Cl_2 can be antagonistic. There may be a hint of this antagonism for the cells treated with low energy density ultrasound (Figure 10), with a slightly steeper slope for untreated cells in response to Cl_2, but the effect was not significant under the conditions tested.

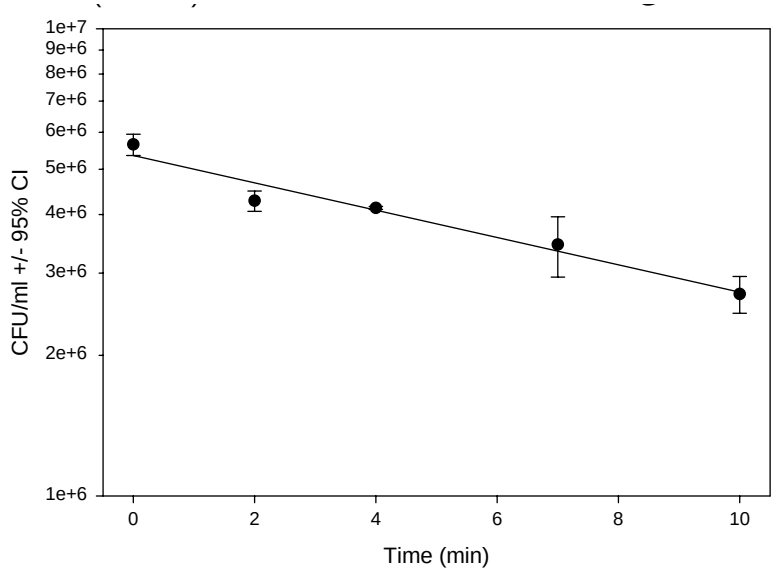

Figure 9. Survival of *V. cholerae* as a function of exposure time to low intensity (12 W·cm-2) ultrasound at low energy density (13 J·ml-1) in the beaker-scale system. The V. cholerae were subsequently exposed to chlorine (see Figure 10).

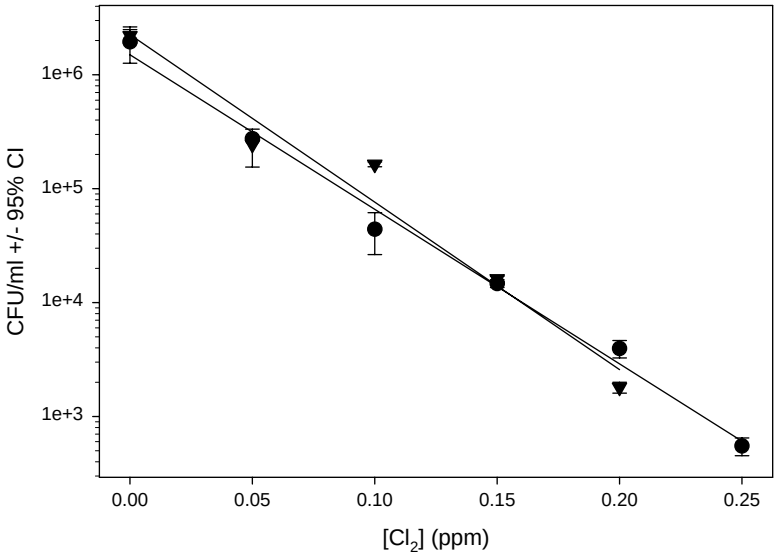

Figure 10. Survival of *V. cholerae* in synthetic seawater treated with ultrasound and dosed with total Cl2 from 0-0.25 mg·l-1. Ultrasound pre-treated (●, from Figure 9) and untreated (▼). All samples were quenched with sodium thiosulfate after 1 minute of Cl2 exposure. The sample not treated by ultrasound and exposed to 0.25 mg·l-1 was less than 500 CFU/ml.

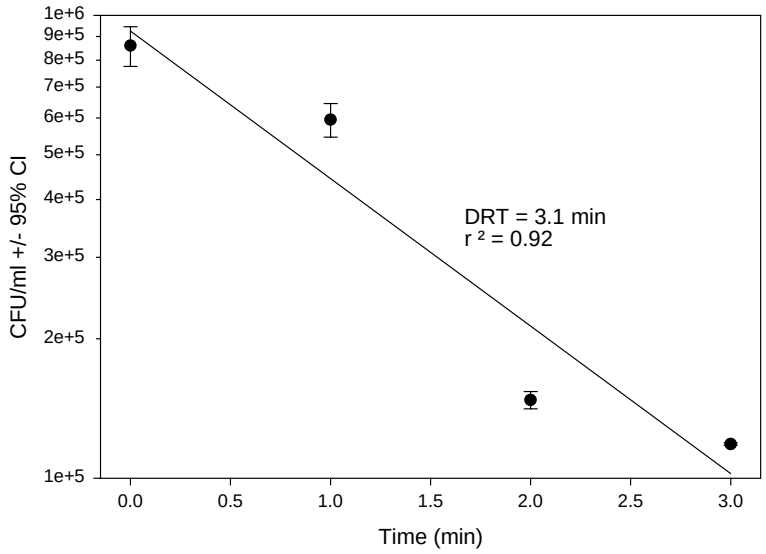

Figure 11. Survival of *V. cholerae* in synthetic seawater as a function of exposure time to high energy density ultrasound. Samples removed at 0, 1, 2 and 3 minutes were subsequently exposed to 0.23 mg $Cl^2 \cdot l^{-1}$ (see Figure 12). The energy density at 3 minute exposure was 68 $J \cdot ml^{-1}$.

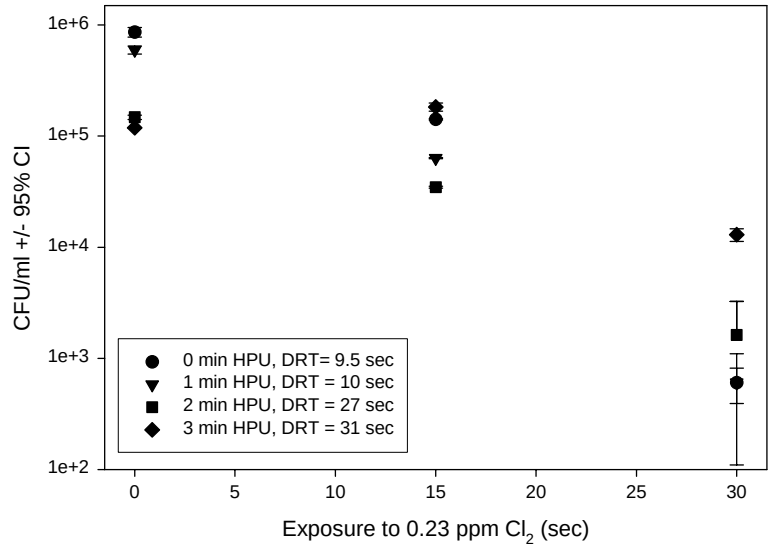

Figure 12. Survival of *V. cholerae* exposed to 0.23 mg $Cl_2 \cdot l^{-1}$ after pretreatment by 30 $W \cdot cm^{-2}$ ultrasound. 0(●), 1(▼), 2(■), and 3(♦) minutes.

DISINFECTION OF ZOOPLANKTON USING COMBINED TREATMENT WITH ULTRASOUND AND HEAT TREATMENT

The mortality rate of *Artemia* was affected by both ultrasonic intensity and temperature. The time required to kill 90% of *Artemia* was lower at higher intensities, and for both intensities, decreased at temperatures greater than 40 °C (Table 2). At low intensity (10 W/cm^2), DRTs at 30°C and 40°C were slightly greater than 2 minutes, and decreased by approximately 1 minute at the high intensity (20 W/cm^2). Increasing the temperature to 43°C resulted in a significant decrease in the DRT at both intensities (Table 2). DRTs reported here are longer than those previously reported (Brizzolara et al., 2006) due to the relatively large liquid volumes used in the present work.

The decrease in DRT at higher temperatures could be due either to an additive effect of temperature and ultrasound, or to synergy between the two. If the effects of temperature and ultrasound were additive, the DRT at 43°C should be similar to a hypothetical DRT value calculated by adding the slope of the mortality curve for 30 – 40°C and the mortality curve for *Artemia* exposed only to temperature stress at 43°C (e.g. Raso et al., 1998). The DRT obtained by combining the data for 30°C and 40°C was 2.2 minutes for the low intensity trials and 1.4 minutes for the high intensity trials. The DRT for the effect of exposure at 43°C was 27.9 minutes (95% confidence interval, 20.9 – 41.7 min). These results suggest that if the combined effects of heat and ultrasound were additive, the hypothetical DRT at 43°C should be 2.1 minutes at low intensity and 1.3 minutes at high intensity. The observed DRT (Table 2) were both significantly lower than these values (*F*-test, $p < .03$ for both intensities). The result indicates that there is significant synergy between ultrasound and heat treatment at relatively low temperatures. Heat and ultrasound combine to produce mortality rates that are significantly higher than would be expected if the effects of the two treatments acted independently.

Table 2. *Artemia sp.* Decimal reduction times (DRT, in minutes) and their 95% confidence intervals (in parentheses) for various temperatures and ultrasonic intensities. Ultrasound and heat were applied simultaneously.

Temperature	10 W/cm^2	20 W/cm^2
30°C	2.3 (2.0-2.6)	1.3 (1.0-1.9)
40°C	2.2 (2.0-2.6)	1.4 (1.2-1.8)
43°C	1.6 (1.4-2.0)	0.8 (0.6-1.2)

CONCLUSIONS

Ultrasound appears feasible for treating zooplankton in shipboard ballast water (Brizzolara et al., 2006). In a previous report, we estimated that it would require a treatment cell volume of 8 gallons and a power consumption of 24 kW per log kill of *Artemia* in a 1000 gpm flow (Brizzolara et al., 2006). We also found that ultrasound is effective in disinfecting bacteria; however, the required contact times are much longer for bacteria than they are for zooplankton and ultrasound requires substantially more energy to kill bacteria relative to zooplankton (Brizzolara et al., 2006). The contact times and energy densities for *E. coli* were deemed not feasible for the flow rates and volumes required for ballast water treatment.

In view of the previous *E. coli* disinfection results, we undertook to examine two approaches for improving the performance of ultrasound in disinfecting bacteria: 1) optimizing the intensity of the ultrasound by optimizing the diameter of the treatment cell and 2) combined treatment approaches for disinfection of bacteria using ultrasound in conjunction with a second treatment. Reducing the diameter of the treatment cell might improve the DRTs for bacteria, by exposing the organisms to a higher average intensity. For bacteria, combined treatment methods employing ultrasound and a second method may offer a route to disinfection of larger water volumes and flow rates. Thermal disinfection damages cells in part by softening the cell wall. Therefore, we investigated whether combining ultrasound and thermal treatment will enhance this effect due to the shear forces the cavitation generates on the cell. This may cause a synergistic disinfection effect between the two treatments. Similarly, we investigated whether application of ultrasound prior to treatment with chlorine would provide damage to the cell wall via the cavitation-induced shear forces, increasing the effectiveness of the chlorine. Finally, we investigated the use of ultrasound at two system pressure values.

In the experiments investigating the effect of optimizing the intensity via the treatment cell diameter, a minimum DRT of approximately 0.6 minutes and a minimum DRED of 100 J/ml were measured. These values represent a substantial improvement over previously measured minimum DRT and DRED for *E. coli*, 1.4 minutes and 180 J/ml, respectively. Reductions in DRT of *E. coli* were also observed for combined treatment consisting of ultrasound and thermal treatment. The DRT and DRED (ultrasonic energy density only; thermal energy not included) were 1.2 minutes and 90 J/ml at 30^{o}C and 0.8 minutes and 50 J/ml at 45^{o}C. These experiments were performed at a non-optimal treatment cell diameter. Ultrasonic and thermal treatment of *E. coli* at 45^{o}C using the smallest diameter treatment cell might result in a DRT somewhat lower than 0.6 minutes. Additionally, further reduction of treatment cell diameter or increase in ultrasonic intensity might result in further reduced DRTs, based on the fact that no plateau was observed in the treatment cell diameter versus DRT relationship, for the range of treatment cell diameters investigated. For a combined treatment approach consisting of ultrasound and chlorination, no significant difference was found in *V. cholerae* mortality produced by chlorination between cultures pretreated by ultrasound and cultures not pretreated by ultrasound. Chlorination is such an effective disinfectant for *V. cholerae*, even if any improvement could be realized by using ultrasound, it would likely not be enough to be of value. No difference in DRT or DRED of *E. coli* was observed as a function of pressure. Finally, for *Artemia*, heat and ultrasound combine to produce mortality rates that are significantly higher than would be expected if the effects of the two treatments acted independently.

These results indicate that providing very high intensity throughout the volume of the treatment cell is critical for bacterial disinfection. The drawback is that this results in lower flow rates since the treatment cell diameter must be reduced. Since a DRT of 0.6 minutes is still likely to be too long for a flow-through treatment system for ballast water given the high flow rate requirements, the applicability of ultrasound to ballast water treatment is expected to be focused on zooplankton, for which ultrasound is very effective. In addition, a second treatment that targets the bacteria could be employed. For thermal treatment as the second treatment, if applied simultaneously with the ultrasound, synergistic effects can be expected for both zooplankton and bacteria, based on the current results. Finally, ultrasound is very effective in disinfecting both bacteria and zooplankton in smaller flow rates and may have applicability for other water treatment applications.

Based on the results of this work, additional experimentation is recommended, using ultrasound to disinfect natural seawater. The efficacy of ultrasound under these conditions against various organisms of concern for non-indigenous species transfer, including zooplankton, phytoplankton and bacteria, should be established.

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